more than 130 percent of the number of units of polymyxin B that it is represented to contain.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *pH.* Proceed as directed in §440.80a(b)(5)(ii) of this chapter, using the undiluted sample.

[39 FR 19045, May 30, 1974, as amended at 41 FR 56307, Dec. 28, 1976; 42 FR 18059, Apr. 5, 1977; 50 FR 19919, May 13, 1985]

PART 446—TETRACYCLINE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.

Subpart B—Oral Dosage Forms

Subpart C—Injectable Dosage Forms

AUTHORITY: Sec. 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357).

SOURCE: 39 FR 19076, May 30, 1974, unless otherwise noted.

Subpart A—Bulk Drugs

§ 446.10 Chlortetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride is [4S - (4α,4aα,5aα,6β, 12aα] - 7 - chloro - 4 - (dimethylamino) - 1,4, 4a,5,5a,6,11,12a - octahydro - 3,6,10,12, 12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamidemonohydrochloride. Chlortetracycline is produced by the growth of *Streptomyces aureofaciens.* It is a yellow powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 3.3.

(v) It is crystalline.

(vi) It meets the identity test for chlortetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.01*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of chlortetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(6) *Identity.* To 1 milligram of sample, add 2.0 milliliters of concentrated sulfuric acid. In the presence of chlortetracycline, a deep blue color is produced that becomes dark green.

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.10a Sterile chlortetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride is [4S - (4α,4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. Chlortetracycline is produced by the growth of *Streptomyces aureofaciens.* It is a yellow powder. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 3.3.

(viii) It is crystalline.

(ix) It meets the identity test for chlortetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, crystallinity, and identity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.01*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of chlortetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams of chlortetracycline hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* To 1.0 milligram of sample, add 2.0 milliliters of concentrated sulfuric acid. In the presence of chlortetracycline, a deep blue color is produced that becomes dark green.

[43 FR 11154, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.15 Demeclocycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline is [4S - (4α,4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,6,10,12,12a - pentahydroxy - 1, 11 - dioxo - 2 -

naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 970 micrograms of demeclocycline hydrochloride equivalent per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 6.7 percent.

(iv) Its pH is an aqueous solution containing 10 milligrams per milliliter is not less than 4 and not more than 5.5.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the demeclocycline hydrochloride working standard is 107.4±3.88.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10-milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the demeclocycline hydrochloride working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{Weight of sample in milligrams} \times (100 - m)}$$

where: m=percent moisture in the sample.

(6) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(7) *Identity.* Proceed as directed in § 446.16(b)(7). The value yielded by calculation ranges between 0.97 and 1.17.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 16683, Mar. 13, 1981; 50 FR 19920, May 13, 1985]

§446.16 Demeclocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride is [4S - (4α 4aα,5aα,6β,12aα)] - 7 - chloro - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro-3,6,10,12,12a - pentahydroxy - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2 and not more than 3.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the demeclocycline hydrochloride standard is 100±4.2 percent.

(vi) It is crystalline.

(vii) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 250 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of demeclocycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the percent absorptivity of the sample relative to that of the standard in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams of the sample in 2 milliliters of 0.1*N* HCl, dilute to exactly 250 milliliters with distilled water, and mix thoroughly. Transfer a 10 milliliter aliquot of this solution to a 100-milliliter volumetric flask. Add about 75 milliliters of distilled water and 5 milliliters of 5*N* NaOH, dilute to volume with distilled water, and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of the solution at a wavelength of 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Treat a portion of the working standard in the same manner. Determine the percent relative absorptivity of the sample using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Weight of standard in milligrams} \times \text{Potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{Weight of sample in milligrams} \times (100 - m)}$$

where: m=percent moisture in the sample.

(6) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(7) *Identity.* Accurately weigh 40 milligrams of the sample and place into a 200-milliliter volumetric flask. Add 100 milliliters of 0.1*N* HCl and place on a

shaker until the sample is dissolved. Dilute to volume with 0.1*N* HCl and mix well. Transfer a 5-milliliter aliquot of the solution to each of two 50-milliliter volumetric flasks. To one flask add 10 milliliters of 6*N* HCl and to the other add 10 milliliters of 3*N* HCl. Place the acid-treated flasks into a boiling water batch for 20 minutes. Remove the flasks and place in a cold water bath. When cool, dilute to volume with water and mix well. Treat a portion of the standard in the same manner. Using a suitable spectrophotometer, place the 6*N* HCl-treated sample into the reference cell and read against the 3*N* HCl-treated sample at a wavelength of 368 nanometers. Reverse the order of the cells in the cell holder and read at a wavelength of 430 nanometers.

$$\frac{(A_{368}+A_{430}\ \text{sample})\ (\text{milligrams of standard per milliliter})\ (100)}{(A_{368}+A_{430}\ \text{standard})\ (\text{milligrams of sample per milliliter})\ (100-m)} = 0.9 \text{ to } 1.1$$

where: m=percent moisture in the sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 50 FR 19920, May 13, 1985]

§ 446.20 Doxycycline hyclate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate is [4S - 4aα,4aα,5aα,5aα,6α,12aα]- 4 - dimethylamino) -1,4,4a,5,5a,6,11,12a - octahydro- 3,5,10,12,12a-pentahydroxy - 6 -methyl-1,11- dioxo -2 - naphthacenecarboxamide hydrochloridehemiethanolate hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) It contains not less than 82 nor more than 90 percent doxycycline on an ''as is'' basis.

(vi) It gives a positive identity test for doxycycline hyclate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content*—(i) *Equipment*—(*a*) *Sheet* (*chromatographic*). Whatman No. 4 filter paper for chromatography, 15 × 57 centimeters.

(*b*) *Chamber* (*chromatographic*). Square glass chromatography jar, 30 × 30 × 60 centimeters, equipped with 25-centimeter troughs for descending chromatography.

(ii) *Preparation of solutions*—(*a*) *0.05N Methanolic hydrochloric acid.* Dilute 4.2 milliliters of concentrated hydrochloric acid to 1 liter with methanol.

(*b*) *pH 4.2 buffer.* Mix 5.86 volumes of 0.1*M* citric acid with 4.14 volumes of 0.2*M* disodium phosphate.

(*c*) *Chromatographic system.* Mix toluene, pyridine, and pH 4.2 buffer in volumetric proportions of 20:3:10, respectively. Allow the phases to separate. Place the upper phase in the troughs near the top of the chamber. Place the lower phase in the bottom of the chamber. Saturate the atmosphere of the tightly sealed chamber for 24 hours before use by placing white blotters on two opposite sides of the chamber so that their ends are immersed in the lower phase in the bottom of the chamber. Replace the solvent in troughs before the chromatograms are to be developed.

(iii) *Preparation of the doxycycline standard solution.* Accurately weigh about 50 milligrams of the doxycycline working standard into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid. Store in the refrigerator and use within 7 days.

(iv) *Preparation of sample.* Accurately weight about 50 milligrams of the sample into a 5-milliliter volumetric flask and bring to volume with 0.05*N* methanolic hydrochloric acid.

(v) *Preparation of the chromatogram.* Dip the chromatographic sheets into pH 4.2 buffer and lightly blot each sheet between clean nonfluorescing, white blotters. Use separate sheets for the doxycycline standard solution, for each doxycycline sample solution, and for blanks without standard or sample application. Care must be taken so that the moist sheets do not become too dry; a period of 5 to 10 minutes between impregnating the paper and placing it in the chromatographic chamber is usually satisfactory. Evenly apply a 0.100-milliliter aliquot of a doxycycline solution to the origin line of a sheet as a 14-centimeter-long streak. Place the sheets in the chamber and develop them in a descending manner for 2 hours. The doxycycline band should move approximately 12.5 centimeters from the origin line. Remove the sheets from the chamber and air-dry for about 10 minutes.

(vi) *Processing the chromatogram.* Examine each sheet under 366-nanometer ultraviolet light. Outline the fluorescent bands with a pencil. The main marked area should be approximately 10 × 15 centimeters in size. Outline areas on the blank sheet approximately equal in size and in the same locations as those outlined on the standard sheet. Exposure of the sheets to ammonia or other alkaline vapors must be avoided. Cut the marked areas from the sheets and then cut them into approximately 2-centimeter squares. For each sheet, place the squares from each of the following areas into separate 125-milliliter Erlenmeyer flasks: The main doxycycline band of the sample, the main doxycycline band of the standard, all the other bands of the standard, the area of the blank sheet corresponding to the main band of the standard, the other area of the blank sheet corresponding to the other bands of the standard. The time between removing the sheets from the chamber and placing the squares into the Erlenmeyer flasks should be minimal, since excessive drying of the paper can lead to erratic elutions.

(vii) *Elution.* To each flask add 50 milliliters of 0.05*N* methanolic hydrochloric acid and agitate on a reciprocating shaker for 1 hour. Decant the contents of each flask into another flask by pouring through a small funnel fitted with a glass wool plug.

(viii) *Doxycycline standard solution for direct measurement of absorbance.* Pipette a 0.100-milliliter aliquot of the doxycycline standard solution into each of three 125-milliliter Erlenmeyer flasks. Add 50 milliliters of 0.05*N* methanolic hydrochloric acid to each of these flasks.

(ix) *Absorbance measurement.* Using a suitable spectrophotometer and 0.05*N* methanolic hydrochloric acid as the reference solvent, determine the absorbance of each eluate and of each doxycycline standard solution at the absorption maximum at about 349 nanometers.

(x) *Calculation of percent doxycycline in samples.* Calculate as follows:

$$\text{Percent doxycycline} = \frac{(A_u - A_b)(W_s)}{(A_s - A_b)(W_u)} \times \text{Doxycycline content of the working standard}$$

where:

A_u=Absorbance of the eluate from the main doxycycline band of the sample sheet.

A_s=Absorbance of the eluate from the main doxycycline band on the standard sheet.

A_b=Absorbance of the eluate from the area of the blank sheet corresponding to the area of the doxycycline band of the standard sheet.

W_u=Weight in milligrams of sample.

W_s=Weight in milligrams of doxycycline working standard.

(xi) *Recovery of the doxycycline standard from the chromatogram. As follows:*

$$\text{Percent recovery} = \frac{As - Ab}{A_p} \times \frac{100}{F}$$

where:

A_p=Absorbance of the doxycycline standard solution described in paragraph (b)(5)(viii) of this section.

F=The fractional purity of doxycycline standard solution described in paragraph (b)(5)(xii) of this section.

If the recovery of the doxycycline standard from the chromatogram is less than 95 percent, repeat the chromatogram.

(xii) *Determination of the fractional purity of the doxycycline working standard.* Determine F by means of the following equation:

$$\mathrm{F} = 1 - \frac{Ac - Acb}{Ac - Acb + As - Ab}$$

where:

A_c=Absorbance of the eluate from sections of the standard chromatogram containing nondoxycycline 349 nanometers-absorbing contaminants.

A_{cb}=Absorbance of the eluates from the sections of the blank sheets corresponding to those sections of the nondoxycycline-absorbing contaminants of the standard sheets.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.20a Sterile doxycycline hyclate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, equality, and purity.* Sterile doxycycline hyclate is [4S - (4α,4aα,5α,5aα,6α 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide hydrochloride hemiethanolate hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(viii) It contains not less than 82 nor more than 90 percent doxycycline on an ''as is'' basis.

(ix) It gives a positive identity test for doxycycline hyclate.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 12 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in §436.201 of this subchapter.

(7) *pH*. Proceed as directed in §436.202 of this subchapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(8) *Doxycycline content*. Proceed as directed in §446.20(b)(5).

(9) *Identity*. Proceed as directed in §436.211 of this subchapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(10) *Crystallinity*. Proceed as directed in §436.203(a) of this subchapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.21 Doxycycline monohydrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Doxycycline monohydrate is [4S - (4α,4aα,5α,5aα,6α,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, - 12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphtha - cenecarboxamide monohydrate. It is so purified and dried that:

(i) Its potency is not less than 880 micrograms nor more than 980 micrograms of doxycycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.6 percent nor more than 4.6 percent.

(iv) Its pH in an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter is not less than 5.0 nor more than 6.5.

(v) It contains not less than 90 percent nor more than 98 percent doxycycline on an ''as is'' basis.

(vi) It gives a positive identity test for doxycycline monohydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH*. Proceed as directed in §436.202 of this chapter, using an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter.

(5) *Doxycycline content.* Proceed as directed in §446.20(b)(5).

(6) *Identity*. Proceed as directed in §436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.42 Meclocycline sulfosalicylate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Meclocycline sulfosalicylate is the sulfosalicylate salt of 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 620 micrograms of meclocycline per milligram on an ''as is'' basis.

(ii) Its moisture content is not more than 4.0 percent.

(iii) Its pH is in an aqueous suspension containing 10 milligrams per milliliter is not less than 2.5 and not more than 3.5.

(iv) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on potency, moisture, pH, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography method shall be conclusive.

(i) *High-pressure liquid chromatography.* Proceed as directed in § 436.329 of this chapter.

(ii) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient 0.01*N* methanolic hydrochloric acid (solution 13) to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.06 microgram of meclocycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams of meclocycline per milliliter.

(4) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[46 FR 3836, Jan. 16, 1981]

§ 446.50 Methacycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride is [4S - (4α,4aα,5α,5aα, - 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methylene - 1,11 - dioxo - 2 - naphthacenecar - boxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of methacycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(v) Its absorptivity at the absorption maximum of 345 nanometers relative to that of the methacycline working standard similarly treated is 92.4±4 percent.

(vi) It gives a positive result to the identity test for methacycline hydrochloride.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples of the batch: 10 packages, each containing 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock

solution with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams of methacycline per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 100 milliliters of 0.01*N* methanolic hydrochloric acid. Transfer a 10-milliliter aliquot to a 250-milliliter volumetric flask and dilute to volume with 0.01*N* methanolic hydrochloric acid. Using a suitable spectrphotometer and 0.01*N* methanolic hydrochloric acid as the blank, scan the absorption spectrum between the wavelengths of 250 and 400 nanometers. Determine the absorbance of each solution at the maxima, ca. 345 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

Percent relative absorptivity=(Absorbance of sample×weight in milligrams of standards×potency of standard in micrograms per milligram)/(Absorbance of standard×weight in milligrams of sample×10)

(6) *Identity.* The absorption spectrum between the wavelength of 250 and 400 nanometers, determined as directed in paragraph (b)(5) of this section, compares qualitatively with that of the methacycline standard.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§446.60 Minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride is [4S-(4α,4aα,5aα,12aα)]-4,7-bis (dimethylamino)-1,4,4a,5,5a,6,11, - 12a-octahydro-3,10,12, - 12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 890 micrograms per milligram and not more than 950 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 4.3 percent and not more than 8.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams of minocycline per milliliter is not less than 3.5 and not more than 4.5.

(v) Its epi-minocycline content is not more than 1.2 percent.

(vi) It gives a positive identity test for minocycline hydrochloride.

(vii) It is crystalline.

(viii) Its residue on ignition is not more than 0.15 percent.

(ix) The absorptivity at 560 nanometers of an aqueous solution containing 10 milligrams of minocycline hydrochloride per milliliter is not more than 0.006.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Minocycline potency.* Proceed as directed in §436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 4.6-millimeter X 3-centimeter guard column containing 10-micrometer diameter RP–8 Lichrosorb, a 4.6-millimeter X 15-centimeter analytical column packed with octyl silane chemically bonded to porous microsilica particles, 5 micrometers in diameter, a flow rate of 2.9 milliliters per minute, and a known injection volume of 10 microliters. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(*a*) *0.1 M Disodium ethylenediamine-tetraacetate (EDTA).* Accurately weigh 37.22 grams of disodium ethylenediaminetetraacetate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*b*) *0.2 M Ammonium oxalate.* Accurately weigh 28.42 grams of ammonium oxalate into a 1,000-milliliter volumetric flask. Dissolve in and dilute to mark with deionized water.

(*c*) *Mobile phase.* Mix 250 milliliters of dimethylformamide, 200 milliliters of 0.1*M* disodium ethylenediaminetetraacetate and 550 milliliters of 0.2*M* ammonium oxalate. (5:4:11). Allow the solution to cool to room temperature and then adjust the pH to 6.2 to 6.3 with 0.4*M* tetrabutylammonium hydroxide. Filter and degas the mobile phase just prior to its introduction into the chomatographic pumping system.

(ii) *Preparations of working standard, sample and resolution testing solutions*—(*a*) *Working standard solution.* Dissolve an accurately weighed portion of the minocycline hydrocholoride working standard with sufficient mobile phase (prepared as described in paragraph (b)(1)(i)(*c*) of this section) to obtain a solution containing 500 micrograms of minocycline activity per milliliter. Use this standard solution within 3 hours of preparation.

(*b*) *Sample solution.* Dissolve an accurately weighed sample in sufficient mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparations.

(iii) *System suitability requirements*—(*a*) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to point of maximum peak height; and
b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s)is satisfactory if it is not less than 0.9 and not more than 1.35.

(*b*) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in §436.216(c)(2) calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;
n=number of theoretical plates; and
d_p=Average diameter of the particles in analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 50 for the minocycline peak.

(*c*) *Resolution.* Dissolve 50 milligrams of minocycline hydrochloride in 25 milliliters of deionized water. Pipet 5 milliliters of this solution into a 25-milliliter volumetric flask and heat on a steam bath for 60 minutes. Transfer the contents of the flask to a small beaker and evaporate to dryness. Dissolve the residue in mobile phase, transfer to a 25-milliliter volumetric flask, dilute to mark with mobile phase, mix, and filter through Whatman No. 1 filter paper. Use this solution to determine the resolution factor. The resolution (R) between the peaks for minocycline and epi-minocycline is satisfactory if it is not less than 2.0.

(*d*) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(*e*) *Capacity factor (k′).* Calculate the capacity factor (*k′*) for minocycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of minocycline in minutes; and
t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;
L=Column length in centimeters;

0.75=Average total column porosity; and
F=Flow rate in milliliters per minute.

The capacity factor (k') for minocycline is satisfactory if it is not less than 6.2 and not more than 11.5.

If the system suitability requirements have been met, then proceed as described in § 436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system. However, the sample preparation described in paragraph (b)(1)(ii)(*b*) of this section should not be changed.

(iv) *Calculations*—Calculate the micrograms of minocycline per milligram of sample as follows:

$$\text{Micrograms of minocycline per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:
A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);
A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;
P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;
C_u=Milligrams of minocycline sample per milliliter of sample solution; and
m=Percent moisture content of the sample.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

(5) *Epi-minocycline content.* Proceed as directed in paragraph (b)(1) of this section. Calculate the epi-minocycline content as follows:

$$\text{Percent Epi-minocycline} = \frac{(A_{epi}) \times 100}{(A_{total})}$$

where:
A_{epi}=Area of the epi-minocycline peak in the chromatogram of the sample; and
A_{total}=The sum of the areas of all the peaks eluting after the solvent front.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 0.5 percent potassium bromide disc prepared as described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter.

(9) *Absorptivity.* Accurately weigh about 1 gram of sample into a 100-milliliter volumetric flask, dissolve, and dilute to mark with deionized water. Determine the absorbance of this solution on a suitable spectrophotometer at 560 nanometers (nm) using 5-centimeter cells with water in the reference cell. Calculate the absorptivity as follows:

$$\text{Absorptivity at 560 nm} = \frac{(A_{560})\ (100)}{(\text{grams of sample})\ (1{,}000)(5)}$$

[39 FR 19076, May 30, 1974, as amended at 43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32607, Aug. 26, 1988; 53 FR 39839, Oct. 12, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.65 Oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline is [4S-(4α,4aα,5α,5aα,6β,12aα)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 6 percent and not more than 9 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(v) When calculated on an anhydrous basis its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 100±4 percent.

(vi) It gives a positive result to an identity test for oxytetracycline.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.65a Sterile oxytetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile oxytetracycline is [4S - (4α,4aα,5α,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, 12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide dihydrate. Oxytetracycline is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is not less than 832 micrograms of oxytetracycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 6 percent and not more than 9 percent.

(vii) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 4.5 and not more than 7.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytet- racycline working standard similarly treated, is 100±4 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, absorptivity, identity, and crystallinity.

(ii) Samples required:

(a) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milli- liter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of oxytetracycline per milliliter prepared by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile, pyrogen-free distilled water.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter, preparing the sample by dissolving 40 milligrams in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with the required amount of sterile distilled water.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(8) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask, and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11156, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.66 Oxytetracycline calcium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline calcium is [4S-(4α,4aα,5α,5aα,6β,12αβ)]-4-(dimethylamino)-1,4,4a,5,5a,6,11, 12a-octahydro-3,5,6,10,12,12a-hexa hydroxy-6-methyl-1,11-dioxo- 2-naphthacenecarboxamide calcium salt. Oxytetracycline is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is equivalent to not less than 865 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 8 percent and not more than 14 percent.

(iv) Its pH in an aqueous suspension containing 25 milligrams per milliliter is not less than 6.0 and not more than 8.0

(v) Its calcium content as the sulfated ash is not less than 3.85 percent and not more than 4.35 percent on an anhydrous basis.

(vi) It gives a positive identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, calcium content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in § 436.320 of this chapter.

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a saturated aqueous suspension containing 25 milligrams per milliliter.

(5) *Calcium content.* Proceed as directed in § 436.207(b) of this chapter, except from the weight of residue obtained calculate the calcium content as follows:

$$\text{Percent calcium} = \frac{\text{Weight of residue} \times 0.29435 \times 100 \times 100}{\text{Weight of sample (anhydrous basis)} \times (100 - m)}$$

where: m = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[43 FR 11157, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.67 Oxytetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride is [4S-(4α,4aα,5α,5aα, 6β,12aα,)] - 4 - (dimethylamino) - 1,4,4a,5, 5a,6, 11, 12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. Oxytetracycline is

produced by the growth of *Streptomyces rimosus*. It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(v) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytetracycline standard similarly treated is 92.5±4.3 percent.

(vi) It gives a positive result to an identity test for oxytetracycline.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milli- liter (estimated).

(ii) *Chemical assay.* Proceed as directed in §436.320 of this chapter.

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard, using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 11157, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.67a Sterile oxytetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality,*

and purity. Sterile oxytetracycline hydrochloride is [4S - (4α,4aα,5α,5aα,6β,12α)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,6,10,12,12a - hexahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is produced by the growth of *Streptomyces rimosus.* It is so purified and dried that:

(i) Its potency is not less than 835 micrograms of oxytetracycline per milligram on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0.

(viii) When calculated on an anhydrous basis, its absorptivity at 353 nanometers relative to that of the oxytetracycline working standard similarly treated is 92.5±4.3 percent.

(ix) It gives a positive result to an identity test for oxytetracycline.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Assay for potency by either of the following methods; however, the results obtained from the microbiological turbidimetric assay shall be conclusive.

(i) *Microbiological turbidimetric assay.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Chemical assay.* Proceed as directed in §436.320 of this chapter.

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a solution containing 5 milligrams of oxytetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(7) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve approximately 50 milligrams each of the sample and standard in 250 milliliters of 0.1*N* hydrochloric acid. Transfer a 10-milliliter aliquot to a 100-milliliter volumetric flask and dilute to volume with 0.1*N* hydrochloric acid. Using a suitable spectrophotometer and 0.1*N* hydrochloric acid as the blank, determine the absorbance of each solution at 353 nanometers. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{Milligrams of standard}}{\text{Absorbance of standard} \times \text{Milligrams of sample}} \times \text{Potency of standard in micrograms per milligram} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *Identity.* To about 1 milligram of sample, add 2 milliliters of sulfuric acid; a light-red color is produced when oxytetracycline is present.

(10) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.75a Sterile rolitetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile rolitetracycline is [4S-(4α,4aα,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram on the anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not more than 3.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7 and not more than 9, and such solution is substantially clear.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard similarly treated is 100±4.4 percent.

(x) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed portion of the sample in sufficient methyl alcohol to give a solution containing 1 milligram of rolitetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in §436.201 of this subchapter.

(7) *pH.* Proceed as directed in §436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this subchapter.

(9) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water

and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to separate 100-milliliter volumetric flasks. Add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each flask, and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where m=percent moisture in the sample.

(10) *Identity.* Place approximately 100 milligrams of the sample to be tested in a test tube, and 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, aminelike odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11158, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.76a Sterile rolitetracycline nitrate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile rolitetracycline nitrate is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - *N* - (1 - pyrrolidinylmethyl) - 2 - naphthacenecarboxamide mononitrate sesquihydrate. It is so purified and dried that:

(i) It contains not less than 765 micrograms of rolitetracycline per milligram on an ''as is'' basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 3.5 and not more than 5.5.

(viii) It is crystalline.

(ix) When calculated on an anhydrous basis, its absorptivity at 380 nanometers relative to that of the rolitetracycline standard treated is 89.2±4.0 percent.

(x) It gives a positive result to the identity tests for rolitetracycline nitrate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient sterile distilled water to obtain a stock solution of convenient concentration.

Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in §436.201 of this subchapter.

(7) *pH.* Proceed as directed in §436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this subchapter.

(9) *Absorptivity.* Determine the absorbance of the sample and standard solutions in the following manner: Dissolve an accurately weighed portion of approximately 40 milligrams each of the sample and standard in approximately 150 milliliters of distilled water and mix thoroughly. Dilute each to exactly 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of each of these solutions to representative 100-milliliter volumetric flasks. Add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH to each and then dilute to volume with water and mix thoroughly. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample} \times \text{weight of standard in milligrams} \times \text{potency of standard in micrograms per milligram} \times 10}{\text{Absorbance of standard} \times \text{weight of sample in milligrams} \times (100 - m)}$$

where: m=percent moisture in the sample.

(10) *Identity*—(i) *Rolitetracycline.* Place approximately 100 milligrams of the sample to be used in a test tube, add 5 milliliters of 1*N* NaOH, and heat gently to boiling for about 15 seconds. (The musty, amine-like odor of pyrrolidine is detectable.) Allow to cool to room temperature. A deep burgundy-red color of the clear solution indicates the presence of rolitetracycline.

(ii) *Nitrate identity.* Transfer approximately 1 gram of sample to a 250-milliliter beaker, add 100 milliliters of water, and acidify with 1 milliliter of acetic acid. Heat to boiling and, with constant stirring, add 10 milliliters of a 10-percent solution of nitron (1,4-diphenyl-3,5-endo-anilino-4,5-dihydro-1,2,4-triazole) $C_{20}H_{16}N_4$[7] in 1*N* acetic acid. Allow to cool. A heavy precipitate indicates the presence of nitrate.

[7] Nitron is available from J. T. Baker Laboratory Chemicals, North Phillipsburg, N.J. 08865.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11159, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.80 Tetracycline.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline is [4S - (4α,4aα,5aα,6β,12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide. It is so purified and dried that:

(i) Its potency is not less than 975 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 13 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 3.0 and not more than 7.0.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 108.2±3.75 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It is crystalline.

(viii) It passes the identity test for tetracycline.

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, each package shall bear on its label or labeling the statement ''For use only in the manufacture of nonparenteral drugs.''

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetra- cycline content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 60 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous suspension containing 10 milligrams per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample (as the anhydrous compound), accurately weighed, in 2.0 milliliters of 0.1*N* hydrochloric acid and dilute with distilled water to 250 milliliters. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(8) *Identity.* Proceed as directed in § 436.308 of this chapter.

[43 FR 11159, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.81 Tetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 1.8 and not more than 2.8.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 100±4 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It is crystalline.

(viii) It passes the identity test for tetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(4) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample, accurately weighed, in approximately 150 milliliters of distilled water by mixing thoroughly. Dilute to 250 milliliters with distilled water and mix thoroughly. Transfer a 10.0 milliliter aliquot of this solution to a 100-milliliter volumetric flask, add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in §436.309 of this chapter.

(7) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(8) *Identity.* Proceed as directed in §436.308 of this chapter.

[43 FR 11159, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.81a Sterile tetracycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride is [4S - (4α,4aα,5aα,6β, 12aα)] - 4 - dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacene - carboxamide monohydrochloride. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms of tetracycline hydrochloride per milligram. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its loss on drying is not more than 2 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 1.8 and not more than 2.8.

(viii) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 100±4 percent.

(ix) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(x) It is crystalline.

(xi) It passes the identity test for tetracycline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing.

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a stock solution containing 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated); also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this chapter, using a [so–]lution containing 5.0 milligrams of te–[tracycline] hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in §436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

(7) *pH.* Proceed as directed in §436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *Absorptivity.* Dissolve approximately 40 milligrams of the sample, accurately weighed, in approximately 150 milliliters of distilled water by mixing thoroughly. Dilute to 250 milliliters

with distilled water and mix thoroughly. Transfer a 10.0-milliliter aliquot of this solution to a 100-milliliter volumetric flask, add approximately 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of the NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculation:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(9) *4-Epianhydrotetracycline.* Proceed as directed in §436.309 of this chapter.

(10) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

(11) *Identity.* Proceed as directed in §436.308 of this chapter.

[43 FR 11160, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 44 FR 31636, June 1, 1979; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.82 Tetracycline phosphate complex.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex is [4S-(4α,4aα,5aα,6β, 12aα)] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,6,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacenecarboxamide phosphate complex. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms per milligram on the anhydrous basis.

(ii) [Reserved]

(iii) Its moisture content is not more than 9 percent.

(iv) Its pH in an aqueous suspension containing 10 milligrams per milliliter is not less than 2.0 and not more than 4.0.

(v) When calculated on the anhydrous basis, its absorptivity at 380 nanometers relative to that of the tetracycline hydrochloride working standard similarly treated is 82.0±4.9 percent.

(vi) Its 4-epianhydrotetracycline content is not more than 2.0 percent.

(vii) It passes the identity test, showing a presence of phosphate, a content of not more than 0.2 percent chloride, and a content of not more than 1 percent tetracycline base.

(viii) It is crystalline.

(2) *Labeling.* In addition to the requirements of §432.5 of this chapter, each such package shall bear on its label or labeling the statement ''For use only in the manufacture of nonparenteral drugs''.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, absorptivity, 4-epianhydro tetracycline content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 60 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*N* hydrochloric acid to obtain a concentration of 1,000 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a suspension containing 10 milligrams of the sample per milliliter.

(5) *Absorptivity.* Dissolve approximately 40 milligrams of the sample, accurately weighed, in 2.0 milliliters of 0.1*N* HCl and dilute to 250 milliliters with distilled water. Transfer a 10.0 milliliter aliquot of this solution to a 100-milliliter volumetric flask, add about 75 milliliters of distilled water and 5.0 milliliters of 5*N* NaOH, dilute to volume with water, and mix thoroughly. Treat a sample of the tetracycline hydrochloride working standard in the same manner. Exactly 6 minutes after the addition of NaOH, determine the absorbance of each solution at 380 nanometers, using a suitable spectrophotometer and distilled water as the blank. Determine the percent absorptivity of the sample relative to the absorptivity of the standard using the following calculations:

$$\text{Percent relative absorptivity} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \frac{\text{Milligrams of standard}}{\text{Milligrams of sample}} \times \frac{\text{Potency of standard in micrograms per milligram}}{} \times \frac{10}{100 - m}$$

where: m = Percent moisture in the sample.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(7) *Identity*—(i) *Presence of phosphate.* Prepare a filtrate as follows: Suspend 100 milligrams of the sample in 10 milliliters of distilled water and filter a small portion by gravity. Transfer 1.0 milliliter of the filtrate to a 100-milliliter glass-stoppered cylinder, add 10.0 milliliters of distilled water, 2.0 milliliters of ammonium molybdate test solution, 1.0 milliliter of stannous chloride test solution, and 10.0 milliliters of isobutyl alcohol-benzene mixture (1:1 ratio), all in the order named. Shake vigorously for 1 minute, allow the layers to separate, and examine the top organic layer. In the presence of phosphate, the top layer turns blue.

(ii) *Chloride content.* To 1.0 milliliter of the filtrate prepared as directed in the first sentence of paragraph (b)(7)(i) of this section, add 1 drop of silver nitrate test solution and 1 drop of nitric acid. Any turbidity produced is not greater than that obtained by similarly treating 1.0 milliliter of 0.057*N* hydrochloric acid.

(iii) *Determination of percent tetracycline base.* This test is used to determine the quantity of tetracycline present as base in mixtures with phosphate salts.

(a) Reagents—(1) 1,4-Dioxane.

(2) Purified dioxane: Pass the dioxane through a column of Amberlite IRA 400 (OH^-) resin or equivalent.

(3) Perchloric acid, 0.01*N:* Dilute 0.84 milliliter of 70 percent perchloric acid to 1,000 milliliters with purified dioxane; standardize at least once every 2 days, as follows: Weigh accurately about 70 milligrams of diphenylguanidine, and dissolve in 50 milliliters of ethyl alcohol in a 250-milliliter flask. Add two drops of methyl red, and titrate with the perchloric acid solution until the yellow color changes to orange. Deduct the volume of the perchloric acid consumed by 50 milliliters of the ethyl alcohol, and calculate the normality. Each 2.113 milligrams of diphenylguanidine is equivalent to 1 milliliter of 0.01*N* perchloric acid.

(4) Methyl red indicator: Dissolve 100 milligrams of methyl red in 100 milliliters of methyl alcohol.

(b) Procedure. Place an accurately weighed 1-gram sample into a 50-milliliter Erlenmeyer flask, add 10.0 milliliters of purified dioxane and shake the mixture manually for about 2 minutes. Allow to settle, decant all the supernatant liquid into a 50-milliliter polyethylene centrifuge tube, cover with Parafilm (or equivalent), and centrifuge until clear (about 3 minutes). Pipette 5.0 milliliters of the clear, supernatant solution into a 50-milliliter beaker, stir magnetically, and titrate with 0.01*N* perchloric acid, using methyl red as the indicator. The endpoint is the last color change to orange when a

drop of titrant is added. Calculate the percent tetracycline base as follows:

$$\text{Percent tetracycline base} = \frac{\text{Milliliters of acid used} \times \text{Normality} \times 0.4445 \times 200}{\text{Weight of sample}}$$

(8) *Crystallinity.* Proceed as directed in §436.203(a) of this chapter.

[43 FR 11161, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart B—Oral Dosage Forms

§446.110 Chlortetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride capsules are composed of chlortetracycline hydrochloride and one or more suitable and harmless diluents, lubricants, and fillers. Each capsule contains 50, 100, or 250 milligrams of chlortetracycline hydrochloride. The potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. The loss on drying is not more than 1 percent. The chlortetracycline hydrochloride used conforms to the standards prescribed by §446.10(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Test and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.01*N* hydrochloric acid to give a stock solution of convenient concentration. Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[43 FR 11162, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.115 Demeclocycline oral dosage forms.

§446.115a Demeclocycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives suspended in a suitable and harmless vehicle. Each milliliter contains demeclocycline equivalent to 15 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. The pH is not less than 4 and not more than 5.8. The demeclocycline used conforms to the standards prescribed by §446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Mix well. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 43 FR 50677, Oct. 31, 1978; 50 FR 19920, May 13, 1985]

§ 446.115b Demeclocycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline for oral suspension is composed of demeclocycline with or without one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains demeclocycline equivalent to 15 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of demeclocycline hydrochloride equivalent that it is represented to contain. Its moisture content is not more than 5 percent. The demeclocycline used conforms to the standards prescribed by § 446.15(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline used in making the batch for potency, moisture, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The demeclocycline used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.116 Demeclocycline hydrochloride oral dosage forms.

§ 446.116a Demeclocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride tablets are composed of demeclocycline hydrochloride with one

or more suitable and harmless diluents, lubricants, binders, and flavorings. Each tablet contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent. It shall disintegrate within 30 minutes. The demeclocycline hydrochloride used conforms to the standards prescribed by § 446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, and disintegration time.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 436.212 of this chapter.

[39 FR 19076 , May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.116b [Reserved]

§ 446.116c Demeclocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Demeclocycline hydrochloride capsules are composed of demeclocycline hydrochloride, with one or more suitable and harmless diluents and lubricants, enclosed in a gelatin capsule. Each capsule contains 75 milligrams, 150 milligrams, or 300 milligrams of demeclocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of demeclocycline hydrochloride that it is represented to contain. Its loss on drying is not more than 2 percent, except that if starch is used as a diluent the loss on drying is not more than 8 percent. The demeclocycline hydrochloride used conforms to the standards prescribed by § 446.16(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The demeclocycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency and loss on drying.

(ii) Samples required:

(*a*) The demeclocycline hydrochloride used in making the batch: 10 packages, each containing approximately 250 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed

glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of demeclocycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of demeclocycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11162, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§ 446.120 Doxycycline hyclate oral dosage forms.

§ 446.120a Doxycycline hyclate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate capsules are composed of doxycycline hyclate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline hyclate equivalent to either 50, 100, or 300 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.0 percent. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline capsule contents and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final standard and sample solutions. The standard and sample must each produce a major, yellow fluorescent spot with the same R_f value, and the standard-sample mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974. Redesignated at 39 FR 41250, Nov. 26, 1974, and amended at 43 FR 11162, Mar. 17, 1978; 44 FR 20667, Apr. 6, 1979; 50 FR 19920, May 13, 1985]

§ 446.120b Doxycycline calcium oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline calcium oral suspension is prepared from doxycycline hyclate and contains one or more suitable and harmless buffer substances, preservatives, diluents, solvents, colorings, and flavorings. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of

doxycycline that it is represented to contain. Its pH is not less than 6.5 and not more than 8.0. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an appropriate aliquot of the suspension to a volumetric flask and dissolve with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration (containing not less than 150 micrograms of doxycycline per milliliter in acid). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(3) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline calcium oral suspension and of the doxycycline working standard in methanol and further dilute each solution with methanol to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value, and the sample-standard mixed solution must show no separation of major spots.

[39 FR 41250, Nov. 11, 1974, as amended at 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.120c Doxycycline hyclate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate tablets contain doxycycline hyclate with or without one or more disintegrants, lubricants, colorings, and coating substances. Each tablet contains doxycycline hyclate equivalent to 50 or 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. Its moisture content is not more than 5.0 percent. It passes the dissolution test. It passes the identity test. The doxycycline hyclate conforms to the standards prescribed by § 446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, dissolution, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 100 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing

not less than 150 micrograms of doxycycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter, except:

(i) In lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test; and

(ii) In lieu of paragraph (d) of that section, use the interpretation described in the United States Pharmacopeia XX dissolution test. The quantity, *Q* (the amount of doxycycline dissolved) is 55 percent at 60 minutes and 85 percent at 90 minutes.

(4) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the sample and standard solutions as follows: Grind tablet to a powder. Dissolve precise amount of the doxycycline tablet and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final standard and sample solutions. The standard and sample must each produce a major, yellow fluorescent spot with the same R_f value and the standard-sample mixed solution must show no separation of major spots.

[46 FR 7273, Jan. 23, 1981, as amended at 48 FR 23813, May 27, 1983; 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.120d Doxycycline hyclate pellet-filled capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate pellet-filled capsules contain pellets which are composed of doxycycline hyclate and suitable and harmless diluents, binders, and lubricants. Each capsule contains doxycycline hyclate equivalent to 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture content is not more than 5.0 percent. It passes the acid resistance test. It passes the dissolution test. The doxycycline hyclate conforms to the standards prescribed by § 446.20(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, safety, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, acid resistance, and dissolution.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *Acid resistance.* Proceed as directed in § 436.543 of this chapter.

(4) *Dissolution.* Empty the contents of one pellet-filled capsule into the basket and proceed as directed in § 436.544 of this chapter. The quantity *Q* (the

amount of doxycycline dissolved) is 85 percent at 30 minutes.

[50 FR 41679, Oct. 15, 1985, as amended at 55 FR 11584, Mar. 29, 1990]

§ 446.121 Doxycycline monohydrate oral dosage forms.

§ 446.121a Doxycycline monohydrate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate for oral suspension is doxycycline monohydrate with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. Its moisture content is not more than 3 percent. It passes the identity test for the presence of the doxycycline moiety. When prepared as directed in the labeling, each milliliter contains the equivalent of 5 milligrams of doxycycline and its pH is not less than 5.0 and not more than 6.5. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of doxycycline that it is represented to contain. The doxycycline monohydrate used conforms to the standards prescribed by § 446.21(a)(1).

(2) *Labeling.* In addition to the labeling requirements of § 432.5 of this chapter, this drug shall be labeled ''doxycycline for oral suspension''.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline monohydrate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, moisture, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of doxycycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter, using the undiluted sample.

(4) *Identity.* Proceed as directed in § 436.308 of this chapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline monohydrate for oral suspension and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 50 FR 19920, May 13, 1985. Redesignated at 55 FR 6637, Feb. 26, 1990]

§ 446.121b Doxycycline monohydrate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline monohydrate capsules are composed of doxycycline monohydrate and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains doxycycline monohydrate equivalent to 100 milligrams of doxycycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. The moisture

content is not more than 5.5 percent. It passes the dissolution test. It passes the identity test. The doxycycline monohydrate used conforms to the standards prescribed by § 446.21.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(A) The doxycycline monohydrate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(B) The batch for potency, moisture, dissolution, and identity.

(ii) Samples, if required by the Center for Drug Evaluation and Research:

(A) The doxycycline monohydrate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(B) The batch: A minimum of 100 capsules.

(b) *Tests and methods of assay*—(1) *Doxycycline potency.* Proceed as directed in § 436.216 of this chapter, using ambient temperature, an ultraviolet detection system operating at a wavelength of 280 nanometers, a 4.6-millimeter X 3-centimeter guard column containing 5- to 10-micrometer diameter octyl silane chemically bonded to totally porous microsilica particles, a 3.9-millimeter X 30-centimeter analytical column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, a flow rate of 1.5 milliliters per minute, and a 10-microliter loop injector. Reagents, working standard and sample solutions, system suitability requirements, and calculations are as follows:

(i) *Reagents*—(A) *0.1M sodium phosphate buffer.* Prepare a solution containing 13.8 grams of monobasic sodium phosphate per liter of distilled water.

(B) *Mobile phase.* Mix 450 milliliters of 0.1*M* monobasic sodium phosphate and 550 milliliters of methanol. Add 3 milliliters of *N,N*-dimethyl-*n*-octylamine. Adjust the pH to 8.0 with 5*N* sodium hydroxide. Filter the mobile phase through a suitable glass filter or equivalent that is capable of removing particulate contamination to 1 micron in diameter. Degas the mobile phase just prior to its introduction into the chromatograph pumping system.

(ii) *Preparation of working standard, sample, and resolution test solutions*—(A) *Working standard solution.* Dissolve an accurately weighed portion of the doxycycline hyclate working standard in sufficient 0.1*N* hydrochloric acid to obtain a known concentration of about 1,000 micrograms of doxycycline per milliliter. Further dilute with distilled water to a concentration of 40 micrograms of doxycycline activity per milliliter. Filter through a membrane filter of 0.5 micron or finer porosity.

(B) *Sample solution.* Remove, as completely as possible, the contents of a representative number of capsules. Mix the combined contents and transfer an accurately weighed portion of the powder, equivalent to about 100 milligrams of doxycycline, to a 100-milliliter volumetric flask. Add 20 milliliters of 0.1*N* hydrochloric acid and sonicate for 5 minutes. Dilute to mark with 0.1*N* hydrochloric acid. Further quantitatively dilute an aliquot of this solution with distilled water to a concentration of 40 micrograms of doxycycline activity per milliliter (estimated). Filter through a membrane filter of 0.5 micron or finer porosity. Content uniformity analyses may be obtained from sample solutions prepared as above except that the contents of one capsule are quantitatively transferred to the 100-milliliter volumetric flask.

(C) *Resolution test solution.* Dissolve 50 milligrams of doxycycline in 25 milliliters of distilled water. Pipet 5 milliliters of this solution into a 25-milliliter volumetric flask and heat on a steam bath for 60 minutes. Transfer the contents of the flask to a small beaker and evaporate to dryness. Dissolve the residue in distilled water, transfer to a 25-milliliter volumetric flask, dilute to mark with distilled water, mix, and filter through Whatman No. 1 filter paper. Use this solution to determine the resolution factor.

(iii) *System suitability requirements*—(A) *Asymmetry factor.* Calculate the asymmetry factor (A_s), measured at a point 5 percent of the peak height from the baseline, as follows:

$$A_s = \frac{a+b}{2a}$$

where:

a=Horizontal distance from point of ascent to a point of a maximum peak height; and

b=Horizontal distance from the point of maximum peak height to point of descent.

The asymmetry factor (A_s) is satisfactory if it is not less than 1.4 and not more than 2.0

(B) *Efficiency of the column.* From the number of theoretical plates (*n*) calculated as described in §436.216(c)(2) of this chapter calculate the reduced plate height (h_r) as follows:

$$h_r = \frac{(L)(10{,}000)}{(n)(d_p)}$$

where:

L=Length of the column in centimeters;

n=number of theoretical plates; and

d_p=Average diameter of the particles in analytical column packing in micrometers.

The absolute efficiency (h_r) is satisfactory if it is not more than 37.5 for the doxycycline peak.

(C) The resolution (*R*) between peaks for doxycycline and epi-doxycycline is satisfactory if it is not less than 1.5.

(D) *Coefficient of variation (relative standard deviation).* The coefficient of variation (S_R in percent) of 5 replicate injections is satisfactory if it is not more than 2.0 percent.

(E) *Capacity factor (k′).* Calculate the capacity factor (*k′*) for doxycycline as follows:

$$k' = \frac{t_r - t_o}{t_o}$$

where:

t_r=Retention time of doxycycline in minutes; and

t_o=Column dead time in minutes, which is estimated from the following equation:

$$t_o = \frac{(3.1416)(D^2)(L)(0.75)}{4F}$$

where:

D=Column diameter in centimeters;

L=Column length in centimeters;

0.75=Average total column porosity; and

F=Flow rate in milliliters per minute.

The capacity factor (*k′*) for doxycycline is satisfactory if it is not less than 1.5 and not more than 2.5. If the system suitability requirements have been met, then proceed as described in §436.216(b) of this chapter. Alternate chromatographic conditions are acceptable provided reproducibility and resolution are comparable to the system described. However, the sample preparation described in paragraph (b)(1)(ii)(B) of this section should not be changed.

(iv) *Calculations.* Calculate the doxycycline content as follows:

$$\text{Milligrams of doxycycline per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u=Area of the doxycycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the doxycycline peak in the chromatogram of the working standard;

P_s=Doxycycline activity in the doxycycline working standard solution in micrograms per milliliter;

d=Dilution factor of the sample; and

n=Number of capsules in the sample assayed.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Dissolution.* Proceed as directed in §436.215 of this chapter. The quantity *Q* (the amount of doxycycline dissolved) is 85 percent at 60 minutes.

(4) *Identity.* The high-pressure liquid chromatogram of the sample determined in paragraph (b)(1) of this section compares qualitatively to that of the doxycycline working standard.

[55 FR 6637, Feb. 26, 1990]

§446.150 Methacycline hydrochloride oral dosage forms.

§446.150a Methacycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride capsules are composed of methacycline hydrochloride and one or more suitable and harmless lubricants and diluents

enclosed in a gelatin capsule. Each capsule contains methacycline hydrochloride equivalent to either 70 milligrams of methacycline, 140 milligrams of methacycline, or 280 milligrams of methacycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of methacycline that it is represented to contain. The moisture content is not more than 7.5 percent. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Blend a representative number of capsules in a high-speed glass blender jar containing sufficient sterile distilled water to give a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 50 FR 19920, May 13, 1985]

§ 446.150b Methacycline hydrochloride oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Methacycline hydrochloride oral suspension contains methacycline hydrochloride and one or more suitable and harmless buffers, dispersants, diluents, colorings, flavorings, and preservatives. It contains methacycline hydrochloride equivalent to 14 milligrams of methacycline per milliliter. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of methacycline that it is represented to contain. Its pH is not less than 6.5 nor more than 8.0. The methacycline hydrochloride used conforms to the standards prescribed by § 446.50(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The methacycline hydrochloride used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required.

(*a*) The methacycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask, and dilute to volume with sterile distilled water. Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.06 microgram of methacycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

§446.160 Minocycline hydrochloride oral dosage forms.

§446.160a Minocycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride tablets are composed of minocycline hydrochloride and one or more suitable and harmless diluents, binders, lubricants, coloring, and coating substances. Each tablet contains minocycline hydrochloride equivalent to 100 milligrams of minocycline. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The tablets disintegrate within 30 minutes. The minocycline hydrochloride used conforms to the standards prescribed by §446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Grind a representative number of tablets in a mortar and pestle. Wash the ground tablets into a volumetric flask containing mobile phase (described in §446.60(b)(1)(i)(*c*) of this part) and shake to dissolve. Dilute with mobile phase to give a stock solution of convenient concentration. Filter the stock solution. Further dilute using mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations.* Calculate the minocycline content as follows:

$$\frac{\text{Milligrams of minocycline}}{\text{per milliliter}} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times 5}$$

where:

A_u=Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the minocycline peak in the chromatogram of the minocycline working standard;

P_s=Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of tablets in the sample assayed.

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the procedure described in paragraph (e)(1) of that section.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§446.160b Minocycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride capsules are composed of minocycline hydrochloride and one or more suitable and harmless lubricants and diluents enclosed in a gelatin capsule. Each capsule contains minocycline hydrochloride equivalent to 50 or 100 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of minocycline that it is represented to contain. Its moisture content is not more than 12 percent. The minocycline hydrochloride used conforms to the standards prescribed by §446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Open a representative number of capsules and empty the contents into a volumetric flask containing mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) and shake to dissolve. Dilute with mobile phase to give a stock solution of convenient concentration. Filter the stock solution. Remove an aliquot of the stock solution and further dilute with mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations.* Calculate the minocycline content as follows:

$$\text{Milligrams of minocycline per capsule} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u = Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s = Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s = Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample; and

n = Number of capsules in the sample assayed.

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§ 446.160c Minocycline hydrochloride oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Minocycline hydrochloride oral suspension is minocycline hydrochloride with one or more suitable flavorings, wetting agents, preservatives, and diluents in an aqueous vehicle. Each milliliter contains minocycline hydrochloride equivalent to 10 milligrams of minocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 130 percent of the number of milligrams of minocycline that it is represented to contain. Its pH is not less than 7.0 and not more than 9.0. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Transfer an accurately measured 5-milliliter portion of the well-shaken suspension to a 100-milliliter volumetric flask. Dilute to mark with mobile phase (described in

§446.60(b)(1)(i)(*c*) of this part) and mix well. Filter this solution and use within 3 hours of its preparation.

(ii) *Calculations.* Calculate the minocycline content as follows:

$$\text{Milligrams of minocycline per milliliter} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times 5}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(2) *pH.* Proceed as directed in §436.202 of this subchapter, using the undiluted sample.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11163, Mar. 17, 1978; 44 FR 22058, Apr. 13, 1979; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988]

§446.165 Oxytetracycline oral dosage forms.

§446.165a Oxytetracycline tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline tablets are tablets composed of oxytetracycline and one or more suitable and harmless, diluents, binders, lubricants, colorings, and coating substances. The potency of each tablet is 250 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. The moisture content is not more than 7.5 percent. They shall disintegrate within 1 hour. The oxytetracycline used conforms to the standards prescribed by §446.65(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Moisture.* Proceed as directed in §436.201 of this chapter.

(3) *Disintegration time.* Proceed as directed in §436.212 of this chapter, using the method described in paragraph (e)(1) of that section.

[43 FR 11163, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§§446.165b—446.165c [Reserved]

§446.165d Oxytetracycline for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline for oral suspension is oxytetracycline with one or more suitable and harmless buffer substances, preservatives, diluents, colorings, and flavorings. When prepared as directed in the labeling, each milliliter contains 50 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and

not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its loss on drying is not more than 2 percent. When reconstituted as directed in the labeling, its pH is not less than 5.5 and not more than 7.5. The oxytetracycline used conforms to the standards prescribed by § 446.65(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Mix well. Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *pH.* Reconstitute as directed in the labeling and proceed as directed in § 436.202 of this chapter.

[43 FR 11164, Mar. 17, 1978, as amended at 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.166 Oxytetracycline calcium oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline calcium oral suspension contains oxytetracycline calcium with one or more suitable and harmless buffer substances, suspending and stabilizing agents, flavorings, colorings, solvents, and preservatives suspended in a suitable and harmless vehicle. It may contain *N*-acetyl glucosamine. Each milliliter contains a quantity of oxytetracycline calcium equivalent to 25 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its pH is not less than 5.0 and not more than 8.0. The oxytetracycline calcium used conforms to the standards prescribed by § 446.66(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline calcium used in making the batch for potency, moisture, pH, calcium content, identity, and crystallinity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The oxytetracycline calcium used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the sample to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of

0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted sample.

[43 FR 11164, Mar. 17, 1978 as amended at 43 FR 50677, Oct. 31, 1978; 45 FR 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.167 Oxytetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride capsules are gelatin capsules containing oxytetracycline hydrochloride with or without one or more suitable and harmless buffers, preservatives, diluents, binders, and lubricants. They may contain glucosamine hydrochloride. Each capsule contains 50 milligrams, 100 milligrams, 125 milligrams, or 250 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. The loss on drying is not more than 5.0 percent. It passes the dissolution test. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, loss on drying, and dissolution.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter, except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of oxytetracycline dissolved) is 60 percent within 30 minutes and 85 percent within 60 minutes.

[43 FR 11164, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 3, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§ 446.180 Tetracycline oral dosage forms.

§§ 446.180a—446.180b [Reserved]

§ 446.180c Tetracycline oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline oral suspension is composed of tetracycline with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, and preservatives, suspended in a suitable and harmless vehicle. Each milliliter contains tetracycline equivalent to 25 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its pH is not less than 3.5 and not more than 6.0. Its 4-epianhydrotetracycline content is not more than 5.0 percent. The tetracycline used conforms to the standards prescribed by § 446.80(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline used in making the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative portion of the well-shaken suspension to an appropriate-sized volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Mix well. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted suspension.

(3) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309(b) of this chapter.

[43 FR 11164, Mar. 17, 1978; 43 FR 34456, Aug. 4, 1978, as amended at 45 FR 16472, 16476, Mar. 14, 1980; 50 FR 19920, May 13, 1985]

§ 446.181 Tetracycline hydrochloride oral dosage forms.

§§ 446.181a—446.181c [Reserved]

§ 446.181d Tetracycline hydrochloride tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride tablets contain tetracycline hydrochloride with or without one or more buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings. Each tablet contains 250 milligrams or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 3.0 percent. It passes the dissolution test. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, dissolution, and 4-epianhydrotetracycline content.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 36 tablets.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *Dissolution.* Proceed as directed in § 436.215 of this chapter, except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of tetracycline hydrochloride dissolved) is 60 percent within 30 minutes and 85 percent within 60 minutes.

(4) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11165, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 8, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§ 446.181e Tetracycline hydrochloride capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride capsules are composed of tetracycline hydrochloride with or without one or more suitable and harmless buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings enclosed in a gelatin capsule. Each capsule contains 50, 100, 125, 250, or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 4 percent. Its 4-epianhydrotetracycline content is not more than 3.0 percent. It passes the dissolution test. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, loss on drying, 4-epianhydrotetracycline content, and dissolution.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

(4) *Dissolution.* Proceed as directed in § 436.215 of this chapter except in lieu of paragraph (a) of that section, a distance of 4.5±0.5 centimeters should be maintained between the lower edge of the stirring blade and the lowest inner surface of the vessel during the test. The quantity *Q* (the amount of tetracycline hydrochloride dissolved), except for the 500-milligram capsule, is 60 percent within 30 minutes and 85 percent within 60 minutes. For the 500-milligram capsule, the quantity *Q* is 50 percent within 30 minutes, 70 percent within 60 minutes, and 85 percent within 90 minutes.

[43 FR 11166, Mar. 17, 1978, as amended at 44 FR 48189, Aug. 17, 1979; 47 FR 32938, July 30, 1982; 48 FR 51293, Nov. 8, 1983; 49 FR 37058, Sept. 21, 1984; 50 FR 19920, May 13, 1985]

§ 446.182 Tetracycline phosphate complex capsules.

(a) *Requirements for certification*— (1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex capsules contain tetracycline phosphate complex with or without one or more buffer substances, preservatives, diluents, binders, lubricants, colorings, and flavorings enclosed in a

gelatin capsule. Each capsule contains tetracycline phosphate complex equivalent to 50, 100, 125, 250, or 500 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its loss on drying is not more than 9.0 percent. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline phosphate complex used conforms to the standards prescribed by § 446.82 (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification, samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline phosphate complex used in making the batch for potency, moisture, pH, absorptivity, 4-epianhydrotetracycline content, identity, and crystallinity.

(b) The batch for potency, loss on drying, and 4-epianhydrotetracycline content.

(ii) Samples required:

(a) The tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of 30 capsules.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of capsules into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(3) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11166, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart C—Injectable Dosage Forms

§ 446.220 Doxycycline hyclate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Doxycycline hyclate for injection is a dry mixture of doxycycline hyclate and a buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of doxycycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 2.0 percent. Its pH when reconstituted as directed in the labeling is not less than 1.8 and not more than 3.3. It passes the identity test for the presence of the doxycycline moiety. The doxycycline hyclate used conforms to the standards prescribed by § 446.20a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification: samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The doxycycline hyclate used in making the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, and identity.

(ii) Samples required:

(*a*) The doxycycline hyclate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 20 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample

for assay as follows: Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents from each container if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the solution thus obtained with sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration (containing not less than 150 micrograms of doxycycline in acid). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(a) of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using the drug reconstituted as directed in the labeling.

(8) *Identity.* Proceed as directed in § 436.308 of this subchapter, except prepare the standard and sample solutions as follows: Dissolve precise amounts of the doxycycline hyclate for injection and of the doxycycline working standard in methanol and further dilute each solution to a concentration of 1 milligram of doxycycline per milliliter. Prepare the sample-standard mixed solution by mixing equal volumes of the final concentration of the sample and standard solutions. The sample and standard must each produce a major, yellow fluorescent spot with the same R_f value and the sample-standard mixed solution must show no separation of major spots.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11166, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.260 Sterile minocycline hydrochloride.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sterile minocycline hydrochloride is a lyophilized powder of minocycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of minocycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substance. Its moisture content is not more than 3.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.5. The minocycline hydrochloride used conforms to the standards prescribed by § 446.60(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The minocycline hydrochloride used in making the batch for potency, moisture, pH, epi-minocycline content, identity, crystallinity, residue on ignition, and absorptivity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, moisture, and pH.

(ii) Samples required:

(*a*) The minocycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 446.60(b)(1) of this part, except prepare the sample solution and calculate the minocycline potency as follows:

(i) *Sample solution.* Reconstitute as directed in the labeling. Using a suitable hypodermic needle and syringe, remove the withdrawable contents from each container represented as a single-dose container; or if the labeling specifies the amount of potency in a

given volume of the resultant preparation, withdraw an accurately measured representation portion from each container. Dilute the sample thus obtained with sufficient mobile phase (described in § 446.60(b)(1)(i)(*c*) of this part) to give a stock solution of convenient concentration. Filter the stock solution. Further dilute an aliquot of this stock solution with mobile phase to obtain a solution containing 500 micrograms of minocycline activity per milliliter (estimated). Use this solution within 3 hours of preparation.

(ii) *Calculations—(a)* Calculate the minocycline content of the single-dose vial as follows:

$$\text{Milligrams of minocycline per single-dose vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter; and

d = Dilution factor of the sample.

(*b*) Calculate the minocycline content of the multiple-dose vial as follows:

$$\text{Milligrams of minocycline per multiple-dose vial} = \frac{A_u \times P_s \times d}{A_s \times 1{,}000 \times n}$$

where:

A_u= Area of the minocycline peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s= Area of the minocycline peak in the chromatogram of the minocycline working standard:

P_s= Minocycline activity in the minocycline working standard solution in micrograms per milliliter;

d = Dilution factor of the sample.

n = Volume of sample solution assayed.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5 milligrams per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this chapter, using the sample preparation described in paragraph (d)(4) of that section.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams of minocycline per milliliter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11166, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 44 FR 22058, Apr. 13, 1979; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985; 53 FR 32609, Aug. 26, 1988; 54 FR 47205, Nov. 13, 1989]

§ 446.265 Oxytetracycline injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline injection is a solution of oxytetracycline with or without one or more suitable and harmless buffer substances, anesthetics, preservatives, antioxidants, complexing agents, and solvents. Each milliliter contains 50 milligrams or 125 milligrams of oxytetracycline. Its potency is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its pH is not less than 8.0 and not more than 9.0. The oxytetracycline used conforms to the standards prescribed by § 446.65a(a)(1), except sterility, pyrogens, and depressor substances.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline used in making the batch for potency, moisture, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, and pH.

(ii) Samples required:

(*a*) The oxytetracycline used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Transfer an accurately measured representative quantity of the sample to an appropriate-sized volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of oxytetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using the undiluted solution.

[43 FR 11166, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 48 FR 51293, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.267 Oxytetracycline hydrochloride for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline hydrochloride for injection is a dry mixture of oxytetracycline hydrochloride and a suitable buffer substance. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 3.0 percent. Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 1.8 and not more than 2.8. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a(a)(1), except sterility, pyrogens, and depressor substances.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, promptly remove all the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from the container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in 436.32(b) of this chapter, using a solution containing 5.0 milligrams per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter, using the diluent recommended by the manufacturer in the labeling for the drug.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 25 milligrams per milliliter.

[43 FR 11167, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.275 Rolitetracycline injectable dosage forms.

§ 446.275a Rolitetracycline for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline for intravenous use is a dry mixture of rolitetracycline and one or more suitable buffer substances. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by § 446.75a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11167, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.275b Rolitetracycline for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline for intramuscular use is a dry mixture of rolitetracycline and one or more suitable buffer substances and anesthetic agents. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 3.0 and not more than 4.5. The rolitetracycline used conforms to the standards prescribed by §446.75a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline used in making the batch for potency, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in §436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11167, Mar. 17, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.276 Rolitetracycline nitrate injectable dosage forms.

§446.276a Rolitetracycline nitrate for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rolitetracycline nitrate for intravenous use is a dry mixture of rolitetracycline nitrate and one or more suitable buffer substances. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by §446.76a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this subchapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is [repre-]sented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11168, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§ 446.276b Rolitetracycline nitrate for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Rolitetracycline nitrate for intramuscular use is a dry mixture of rolitetracycline nitrate, one or more suitable buffer substances, and lidocaine hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of rolitetracycline that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. When reconstituted as directed in the labeling, its pH is not less than 2.5 nor more than 4.0. The rolitetracycline nitrate used conforms to the standards prescribed by § 446.76a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The rolitetracycline nitrate used in making the batch for potency, depressor substances, moisture, pH, crystallinity, absorptivity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, and pH.

(ii) Samples required:

(*a*) The rolitetracycline nitrate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this subchapter, preparing the sample

for assay as follows: Reconstitute the sample as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient distilled water to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.24 microgram of rolitetracycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in §436.32(b) of this subchapter, using a solution containing 5.0 milligrams of rolitetracycline per milliliter.

(4) *Loss on drying.* Proceed as directed in §436.200(b) of this subchapter.

(5) *pH.* Proceed as directed in §436.202 of this subchapter, using a solution prepared as directed in the labeling.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11168, Mar. 30, 1978; 46 FR 46313, Sept. 18, 1981; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

§446.281 Tetracycline hydrochloride injectable dosage forms.

§446.281a Sterile tetracycline hydrochloride.

The requirements for certification and the tests and methods of assay for sterile tetracycline hydrochloride packaged for dispensing are described in §446.81a.

§446.281c Tetracycline hydrochloride for intramuscular use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for intramuscular use is a dry mixture of tetracycline hydrochloride, magnesium chloride, or magnesium ascorbate and one or more suitable buffer substances, with or without one or more suitable preservatives and anesthetic agents, and with or without one or more suitable solubilizers and stabilizers. Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by §446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, depressor substances, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all the withdrawable contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline

hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[44 FR 31636, June 1, 1979, as amended at 46 FR 60568, Dec. 11, 1981; 47 FR 13326, Mar. 30, 1982; 50 FR 19920, May 13, 1985]

§ 446.281d Tetracycline hydrochloride for intravenous use.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for intravenous use is a dry mixture of tetracycline hydrochloride with one or more suitable and harmless stabilizing agents. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. It contains no depressor substances. Its loss on drying is not more than 5.0 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, 4-epianhydrotetracycline content, cystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, depressor substances, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum for 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawal contents if it is represented as a single dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this chapter.

(6) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(7) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(8) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[44 FR 31636, June 1, 1979, as amended at 46 FR 60568, Dec. 11, 1981; 47 FR 13326, Mar. 30, 1982; 50 FR 19920, May 13, 1985]

§ 446.282 Tetracycline phosphate complex for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline phosphate complex for injection is a dry mixture of tetracycline phosphate complex, magnesium chloride or magnesium ascorbate and one or more suitable buffer substances, with or without one or more suitable preservatives and anesthetic agents, and with or without one or more suitable solubilizers and stabilizers. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. It is nonpyrogenic. Its loss on drying is not more than 5 percent. Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 and not more than 3.0. Its 4-epianhydrotetracycline content is not more than 3.0 percent. The tetracycline phosphate complex conforms to the standards prescribed by § 446.82(a)(1), and it contains no depressor substance.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline phosphate complex used in making the batch for potency, moisture, pH, depressor substances, absorptivity, 4-epianhydrotetracycline content, identity, and crystallinity.

(*b*) The batch for potency, sterility, pyrogens, loss on drying, pH, and 4-epianhydrotetracycline content.

(ii) Samples required:

(*a*) The tetracycline phosphate complex used in making the batch: 10 packages, each containing approximately 300 milligrams, and one package containing approximately 1 gram.

(*b*) The batch: A minimum of 10 immediate containers.

(b) *Tests and methods of assay*—(1) *Potency.* Reconstitute the sample as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all the withdrawable contents if it is represented as a single-dose container; or, if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute the sample thus obtained with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section, except use 50 milligrams in lieu of 300 milligrams of sample.

(3) *Pyrogens.* Proceed as directed in § 436.32(b) of this chapter, using a solution containing 5.0 milligrams of tetracycline hydrochloride per milliliter.

(4) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(5) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(6) *Depressor substances in the tetracycline phosphate complex used in making the batch.* Proceed as directed in § 436.35 of this chapter. Prepare the test solution by dissolving 40 milligrams of sample in 2.0 milliliters of 0.1*N* hydrochloric acid and diluting with sterile distilled water (diluent 3) to the prescribed concentration.

(7) *4-Epianhydrotetracycline.* Proceed as directed in § 436.309 of this chapter.

[43 FR 11168, Mar. 17, 1978, as amended at 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

Subpart D—Ophthalmic Dosage Forms

§ 446.310 Chlortetracycline hydrochloride ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Chlortetracycline hydrochloride ophthalmic ointment contains chlortetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 10 milligrams of chlortetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The chlortetracycline hydrochloride used conforms to the standards prescribed by § 446.10a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(b) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[43 FR 11169, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.367 Oxytetracycline hydrochloride ophthalmic dosage forms.

§ 446.367c Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-hydrocortisone acetate ophthalmic suspension is oxytetracycline hydrochloride and hydrocortisone acetate in a suitable and harmless oil base containing aluminum tristearate. Each milliliter contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and 15 milligrams of hydrocortisone acetate. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of oxytetracycline that it is represented to contain. It is sterile. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67a (a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, and moisture.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of five immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured, representative portion of the sample into a high-speed glass blender jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(2) *Sterility*. Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(2) of that section, except use 0.25 milliliter of the sample in lieu of 1.0 milliliter.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

[43 FR 11169, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.367e Oxytetracycline hydrochloride-polymyxin B sulfate ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity*. Oxytetracycline hydrochloride-polymyxin B sulfate ophthalmic ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each gram contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. It is sterile. Its moisture content is not more than 1 percent. It passes the test for metal particles. The oxytetracycline hydrochloride used conforms to the standards prescribed by §446.67a (a)(1). The polymyxin B sulfate used conforms to the standards prescribed by §448.30a(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin B content, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch:

(1) For all tests except sterility: A minimum of 16 immediate containers.

(2) For sterility testing: 20 immediate containers collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample

into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the ointment and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washings until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Sterility.* Proceed as directed in §436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in §436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in §436.206 of this chapter.

[43 FR 11170, Mar. 17, 1978; 43 FR 34457, Aug. 4, 1978, as amended at 50 FR 19920, May 13, 1985]

§446.381 Tetracycline hydrochloride ophthalmic dosage forms.

§446.381a Tetracycline hydrochloride ophthalmic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ophthalmic ointment contains tetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 10 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. It passes the test for metal particles. The tetracycline hydrochloride used conforms to the standards prescribed by §446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of §432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of §431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency, sterility, moisture, and metal particles.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch:

(1) For all tests except sterility: A minimum of 15 immediate containers.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the

sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of 3 more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot with sterile distilled water to the reference concentration of 0.24 micrograms of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *Metal particles.* Proceed as directed in § 436.206 of this chapter.

[43 FR 11170, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.381b Tetracycline hydrochloride ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ophthalmic suspension contains tetracycline hydrochloride in a suitable and harmless oily base. Each milliliter contains 10 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. It is sterile. Its moisture content is not more than 0.5 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of five immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately measured representative portion of the sample into a high-speed glass blender jar with 1 milliliter polysorbate 80 and sufficient 0.1*N* hydrochloric acid to give a stock solution of convenient concentration containing not less that 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(3) of that section.

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11171, Mar. 17, 1978, as amended at 46 FR 46313, Sept. 18, 1981; 50 FR 19920, May 13, 1985]

Subpart E—Otic Dosage Forms

§ 446.467 Oxytetracycline hydrochloride-polymyxin B sulfate otic ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate otic ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a suitable and harmless ointment base. Each

gram of ointment contains oxytetracycline hydrochloride equivalent to 5 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline hydrochloride content is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, loss on drying, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1.0 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washings until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11171, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart F—Dermatologic Dosage Forms

§ 446.510 Chlortetracycline hydrochloride ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Chlortetracycline hydrochloride ointment contains chlortetracycline hydrochloride and one or more suitable and harmless preservatives in a suitable and harmless ointment base. Each gram contains 30 milligrams of chlortetracycline hydrochloride. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of chlortetracycline hydrochloride that it is represented to contain. Its moisture content is not more than 0.5 percent. The chlortetracycline hydrochloride used conforms to the standards prescribed by § 446.10(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The chlortetracycline hydrochloride used in making the batch for potency, loss on drying, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The chlortetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 60 milligrams.

(*b*) The batch: A minimum of five immediate containers:

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.01*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.01*N* hydrochloric acid. Combine the extractives in a suitable volumetric flask and dilute to volume with 0.01*N* hydrochloric acid. Further dilute an aliquot with sterile distilled water to the reference concentration of 0.06 microgram of chlortetracycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11172, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.542 Meclocycline sulfosalicylate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Meclocycline sulfosalicylate cream contains meclocycline sulfosalicylate in a suitable and harmless cream base. Each gram contains meclocycline sulfosalicylate equivalent to 10 milligrams of meclocycline. Its potency is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of milligrams of meclocycline that it is represented to contain. The meclocycline sulfosalicylate used conforms to the standards prescribed by § 446.42(a)(1).

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The meclocycline sulfosalicylate used in making the batch for potency, moisture, pH, and crystallinity.

(*b*) The batch for potency.

(ii) Samples required:

(*a*) The meclocycline sulfosalicylate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(b) *Tests and methods of assay; potency.* Use either of the following methods; however, the results obtained from the high-pressure liquid chromatography method shall be conclusive.

(1) *Microbiological turbidimetric assay.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar containing sufficient 0.01*N* methanolic hydrochloric acid (solution 13) to obtain a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot of the stock solution with distilled water to the reference concentration of 0.06 microgram of meclocycline per milliliter (estimated).

(2) *High-pressure liquid chromatography.* Proceed as directed in § 436.329 of this chapter, except, prepare the working standard and sample solution and calculate the meclocycline content as follows:

(i) *Preparation of standard solution.* Accurately weigh an amount of working standard equivalent to approximately 25 milligrams of meclocycline into a 50-milliter volumetric flask. Dissolve and dilute to volume with methanol and mix. Transfer exactly 2.0 milliliters of this solution to a 100-milliliter volumetric flask, dilute to volume with mobile phase, and mix.

(ii) *Preparation of sample solution.* Accurately weigh approximately 0.4 to 0.7 gram of sample into a 50-milliliter glass-stoppered centrifuge tube. Add 20 milliliters of methanol and 20 milliliters of 0.025*N* sulfuric acid. Disperse the sample thoroughly by a combination of ultrasonic/vortexing and shaking by hand. Shake for 15 minutes on a wrist action shaker. Quantitatively transfer the contents of the centrifuge tube into a 50-milliliter volumetric flask. Rinse the centrifuge tube with two 5-milliliter portions of methanol and add to the flask. Dilute to volume with methanol and mix. Transfer a portion of the content of the volumetric flask into an appropriate-sized centrifuge tube. Centrifuge for 5 minutes at 2,000 revolutions per minute. Transfer 5.0 milliliters of this solution into a 50-milliliter volumetric flask and dilute to volume with mobile phase and mix. Filter this solution through a 0.5 micrometer filter. Inject the filtrate onto the column as described in § 436.329(e) of this chapter.

(iii) *Calculations.* Calculate the meclocycline content as follows:

$$\text{Meclocycline content of cream in percent} = \frac{A \times 2 \times \text{milligrams of working standard} \times \text{Potency of working standard in micrograms per milligram}}{B \times 100 \times \text{milligrams of sample}}$$

where:

A=Area or peak height of the sample peak (at a retention time equal to that observed for the standard);

B=Area or peak height of the standard peak.

[46 FR 3837, Jan. 16, 1981; 46 FR 21361, Apr. 10, 1981, as amended at 47 FR 22515, May 25, 1982; 50 FR 1504, Jan. 11, 1985]

§ 446.567 Oxytetracycline hydrochloride dermatologic dosage forms.

§ 446.567a [Reserved]

§ 446.567b Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical ointment is oxytetracycline hydrochloride and polymyxin B sulfate in a

suitable and harmless ointment base. Each gram contains oxytetracycline hydrochloride equivalent to 30 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B sulfate content is satisfactory if it is not less than 90 percent and not more than 125 percent of the number of units of polymyxin B that it is represented to contain. Its moisture content is not more than 1 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate conforms to the standards prescribed by § 448.30(a)(1).

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or within the package: Adequate directions under which the layman can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(*c*) The batch for oxytetracycline content, polymyxin B content, and moisture.

(ii) Samples required:

(*a*) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*c*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the ointment and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and dilute to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Weigh accurately 0.5 to 1 gram of the ointment and place into a 15-milliliter centrifuge tube. Add 10 milliliters of peroxide-free ether. Stir until contents are homogeneous and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant ether. Repeat washing and centrifugation steps once more. Add 10 milliliters of acetone, stir until contents are homogeneous, and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant acetone. Repeat acetone wash and centrifugation once more. Continue acetone washing until the yellow color in the residue disappears. Add 3 to 4 drops of polysorbate 80 to the residue and mix well. Gently wash the residue into a 100-milliliter

volumetric flask with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and further dilute with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11172, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.567c Oxytetracycline hydrochloride-polymyxin B sulfate topical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate topical powder is oxytetracycline hydrochloride and polymyxin B sulfate with a suitable filler. Each gram contains 30 milligrams of oxytetracycline and 10,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. The loss on drying is not more than 2.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67. The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* Each package shall bear on its label or labeling, as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(a) The batch mark.

(b) The name and quantity of each active ingredient contained in the drug.

(c) An expiration date that is 12 months after the month during which the batch was certified, unless the use of a longer expiration period has been approved in accordance with the provisions of § 432.5(a)(3) of this chapter.

(ii) On the label of the immediate container or other labeling attached to or within the package, adequate directions for lay use of the drug.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(b) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin content, and loss on drying.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a high-speed glass blender jar with sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline hydrochloride per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin content.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Accurately weigh 1 gram of the powder and place into a 50-milliliter centrifuge tube. Add 15 milliliters of acetone and 1 drop of concentrated hydrochloric acid and stir well. Add 20 milliliters of acetone and centrifuge for 10 minutes at 3,000 revolutions per minute. Decant the supernatant liquid and repeat the acetone-acid extraction once more. Dissolve and dilute the residue with

sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to obtain a stock solution of convenient concentration. Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

[43 FR 11173, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

§ 446.581 Tetracycline hydrochloride dermatologic dosage forms.

§§ 446.581a—446.581b [Reserved]

§ 446.581c Tetracycline hydrochloride for topical solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride for topical solution is a dry mixture of tetracycline hydrochloride, 4-epitetracycline hydrochloride, and sodium bisulfite packaged in combination with a suitable and harmless aqueous vehicle. When reconstituted as directed in the labeling, each milliliter contains 2.2 miligrams of tetracycline hydrochloride. The tetracycline hydrochloride content of the reconstituted solution is satisfactory if it contains not less than 90 percent and not more than 130 percent of the number of milligrams of tetracycline hydrochloride per milliliter that it is represented to contain. The 4-epitetracycline hydrochloride content is satisfactory if it contains not less than 135 percent and not more than 165 percent of the amount of tetracycline hydrochloride in the reconstituted solution at the time of reconstitution. The loss on drying of the dry mixture is not more than 5.0 percent. When reconstituted as directed in the labeling, its pH is not less than 1.9 and not more than 3.5. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81a, except sterility, pyrogens, and histamine. The 4-epitetracycline hydrochloride used conforms to the following standards: It gives a positive identity test for 4-epitetracycline hydrochloride; its 4-epitetracycline content is not less than 70 percent; its total anhydrotetracycline and 4-epianhydrotetracycline content is not more than 2.0 percent; its loss on drying is not more than 6.0 percent; its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.3 and not more than 4.0.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, and crystallinity.

(*b*) The 4-epitetracycline hydrochloride used in making the batch for 4-epitetracycline content and identity, total anhydrotetracycline and 4-epianhydrotetracycline content, loss on drying, and pH.

(*c*) The batch for tetracycline hydrochloride content, 4-epitetracycline hydrochloride content, loss on drying, and pH.

(ii) Samples required:

(*a*) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay of the tetracycline hydrochloride for topical solution*—(1) *Tetracycline hydrochloride content and 4-epitetracycline hydrochloride content.* Proceed as directed in § 436.340 of this chapter.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter, except use the contents of one immediate container.

(3) *pH.* Proceed as directed in § 436.202 of this chapter, using the solution obtained when reconstituted as directed in the labeling.

(c) *Tests and methods of assay of the 4-epitetracycline hydrochloride used in making the batch*—(1) *4-epitetracycline content and identity.* Proceed as directed in paragraph (b)(1) of this section, except in lieu of § 446.581c(b)(1)(iv) prepare the sample by weighing accurately 20 milligrams ±5 milligrams of 4-epitetracycline hydrochloride bulk powder and transfer to a 25-milliliter

volumetric flask. Dissolve with 1.0 milliliter of methyl alcohol and dilute to volume with the buffer solution. Pipet a 2.0-milliliter aliquot to a 10-milliliter volumetric flask and dilute to volume with the buffer solution. Place the column in a suitable support. Place a 100-milliliter graduate under the column. Open the column stopcock, pipet 2.0 milliliters of solution from the 10-milliliter volumetric flask onto the column packing and allow the sample to permeate the column packing. Place a solvent reservoir containing 20 milliliters of benzene on top of the column and begin to collect the eluate (at flow rate of approximately 1 milliliter per minute). When the benzene level reaches the top of the column packing, replace the empty solvent reservoir with a second solvent reservoir containing 60 milliliters of chloroform and continue elution. When the chloroform level reaches the top of the column packing, replace second empty solvent reservoir with a third solvent reservoir containing 50-milliliters of the *n*-butanol:chloroform mixture and replace the 100-milliliter graduate with a 10-milliliter graduate. Collect 8.0 milliliters of eluate. Replace the 10-milliliter graduate with a 50-milliliter low-actinic volumetric flask and continue collecting the eluate containing the 4-epitetracycline fraction until the column runs dry. Determine the absorbance of the 4-epitetracycline eluate as described in paragraph (b)(1)(v) of this section.

Calculate the 4-epitetracycline hydrochloride content of the 4-epitetracycline hydrochloride bulk powder as follows:

$$\text{Percent by weight 4-epitetracycline content} = \frac{A \times 25 \times 10 \times 50 \times 100}{a \times W \times 2 \times 2}$$

Where:

A=Absorbance at 366 nanometers of the low-actinic 50-milliliter volumetric flask.

a=Previously established mean absorptivity of the tetracycline hydrochloride working standard eluates in liters/gram/centimeter with the calculation corrected for potency.

W=Weight of 4-epitetracycline hydrochloride bulk powder in milligrams.

The identity of the 4-epitetracycline hydrochloride is confirmed if the absorbance of the sample after column elution is such that the 4-epitetracycline hydrochloride content is greater than 70 percent by weight.

(2) *Total anhydrotetracycline and 4-epianhydrotetracycline content.* Proceed as directed in § 436.309 of this chapter.

(3) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 10 milligrams per milliliter.

[42 FR 59066, Nov. 15, 1977; 43 FR 3705, Jan. 27, 1978, as amended at 48 FR 51291, Nov. 8, 1983; 50 FR 19920, May 13, 1985]

§ 446.581d Tetracycline hydrochloride ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Tetracycline hydrochloride ointment contains tetracycline hydrochloride in a suitable and harmless ointment base. Each gram contains 30 milligrams of tetracycline hydrochloride. Its potency is satisfactory if it contains not less than 90 percent and not more than 125 percent of the number of milligrams of tetracycline hydrochloride that it is represented to contain. Its moisture content is not more than 1 percent. The tetracycline hydrochloride used conforms to the standards prescribed by § 446.81(a)(1), except 4-epianhydrotetracycline content.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 432.5(a)(3) of this chapter, each package shall bear on its label or labeling as hereinafter indicated, the following:

(i) On the label of the immediate container and on the outside wrapper or container, if any:

(*a*) The batch mark.

(*b*) The name and quantity of each active ingredient contained in the drug.

(ii) On the label of the immediate container or other labeling attached to or inserted within the package: Adequate directions under which the layperson can use the drug safely and efficaciously.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The tetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, crystallinity, and identity.

(b) The batch for potency and moisture.

(ii) Samples required:

(a) The tetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The batch: A minimum of six immediate containers.

(b) *Tests and methods of assay*—(1) *Potency*. Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Place an accurately weighed representative portion of the sample into a separatory funnel containing approximately 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 20 to 25 milliliters of 0.1*N* hydrochloric acid and shake well. Allow the layers to separate. Remove the acid layer and repeat the extraction procedure with each of three more 20- to 25-milliliter quantities of 0.1*N* hydrochloric acid. Combine the acid extractives in a suitable volumetric flask and fill to volume with 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of tetracycline hydrochloride per milliliter (estimated). Further dilute an aliquot of the stock solution with sterile distilled water to the reference concentration of 0.24 microgram of tetracycline hydrochloride per milliliter (estimated).

(2) *Moisture.* Proceed as directed in § 436.201 of this chapter.

[43 FR 11174, Mar. 17, 1978 as amended at 44 FR 30333, May 25, 1979. Redesignated at 45 FR 16472, Mar. 14, 1980, and amended at 50 FR 19920, May 13, 1985]

Subpart G—Vaginal Dosage Forms

§ 446.667 Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oxytetracycline hydrochloride-polymyxin B sulfate vaginal tablets are tablets composed of oxytetracycline hydrochloride and polymyxin B sulfate with one or more suitable diluents, binders, lubricants, and preservatives. Each tablet contains oxytetracycline hydrochloride equivalent to 100 milligrams of oxytetracycline and polymyxin B sulfate equivalent to 100,000 units of polymyxin B. Its oxytetracycline content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of milligrams of oxytetracycline that it is represented to contain. Its polymyxin B content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain. The loss on drying is not more than 3.0 percent. The oxytetracycline hydrochloride used conforms to the standards prescribed by § 446.67(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 448.30(a)(1) of this chapter.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The oxytetracycline hydrochloride used in making the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(b) The polymyxin B sulfate used in making the batch for potency, loss on drying, pH, residue on ignition, and identity.

(c) The batch for oxytetracycline content, polymyxin B content, and loss on drying.

(ii) Samples required:

(a) The oxytetracycline hydrochloride used in making the batch: 10 packages, each containing approximately 300 milligrams.

(b) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(c) The batch: A minimum of 30 tablets.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Oxytetracycline content.* Proceed as directed in §436.106 of this chapter, preparing the sample for assay as follows: Place a representative number of tablets into a high-speed glass blender jar containing sufficient 0.1*N* hydrochloric acid to obtain a stock solution of convenient concentration containing not less than 150 micrograms of oxytetracycline per milliliter (estimated). Blend for 3 to 5 minutes. Remove an aliquot of the stock solution and further dilute with sterile distilled water to the reference concentration of 0.24 microgram of oxytetracycline per milliliter (estimated).

(ii) *Polymyxin B content.* Proceed as directed in §436.105 of this chapter, preparing the sample for assay as follows: Grind a representative number of tablets into a fine powder and place this powder, accurately weighed, into a filter funnel with a solvent-resistant membrane filter of 1.0 micrometer porosity. Wash the powder with five 20-milliliter portions of acetone or until the yellow color has disappeared. Remove the filter and soak in 400 milliliters of 10 percent potassium phosphate buffer, pH 6.0 (solution 6), and blend. Quantitatively transfer to a 500-milliliter volumetric flask and adjust to volume with solution 6. Further dilute an aliquot with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) *Loss on drying.* Proceed as directed in §436.200(b) of this chapter.

[43 FR 11174, Mar. 17, 1978, as amended at 50 FR 19920, May 13, 1985]

Subpart H—Rectal Dosage Forms [Reserved]

Subpart I—[Reserved]

Subpart J—Certain Other Dosage Forms [Reserved]

PART 448—PEPTIDE ANTIBIOTIC DRUGS

Subpart A—Bulk Drugs

Sec.

Subpart B—Oral Dosage Forms

Subpart C—Injectable Dosage Forms

Subpart D—Ophthalmic Dosage Forms